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ANNUAL REPORT

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Principal Investigators: A. Warburg (Israel), Y. T. Touré (Mali).

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Section 1

A. Research Objectives

To identify potential aestivation habitats for *Anopheles gambiae*, evaluate their importance as sources for rainy season "recolonization" and the subsequent resumption of malaria transmission. Results of field studies will also clarify possible aestivation by other important malaria vectors, most notably *An. funestus*.

The following subsidiary objectives were undertaken to achieve this:

1. Devise methods for capturing mosquitoes entering or exiting potential aestivation shelters. Devise methods for extracting aestivating mosquitoes from different types of shelters. Measure the gas composition and temperature inside rodent burrows and other aestivation sites of *An. gambiae*.
2. Characterize populations of aestivating mosquitoes or those attempting to enter or exit shelters during different times of the year in terms of their karyotype, gonotrophic status and *Plasmodium* sporozoite infection rates.
3. Simulate in the laboratory, putative environmental cues for aestivation and study their effects on the physiology of mosquitoes (metabolic rate, gonotrophic cycles, longevity).

B. Research Accomplishments - Mali

During the year to 31/1/00 a number of new sampling techniques were employed in the field and efforts were focussed on capturing *An. gambiae* s.l. during the transition period from dry season to wet (May-Aug). Sampling, however, continued well into the wet season (Sept 1999), by which time all chromosomal forms of *An. gambiae* s.s. are known to be present in the area. Studies concentrated, once again, on the village of Doneguebougou (25km NE of Bamako)

In addition, we addressed the question of a "clustering" effect. This hypothesis suggested that should one habitat exist throughout the dry season that contains suitable aestivation sites, it may well be the focus for a concentration of aestivating *An. gambiae* s.l. One area fitting this criterion was identified and sampled, a small area of permanent standing water in the neighboring village of Banambani.

Great emphasis was placed this year on completing molecular (PCR) and cytotoxic identification of the many insects sampled during the first year of the project. The establishment of a PCR-RFLP diagnostic test at MRTC allows us, for the first time, to distinguish the Mopti chromosomal form from Bamako/Savanna forms.

B.1. Summary of Results

B.1.1. Pyrethrum Spray Catches.

i. Human dwellings - This aspect of the study was continued throughout the reporting period in Doneguebougou. However, PSC sampling in all four villages (Kalaban Coro, Moribabougou, Banambani and Doneguebougou) was discontinued in March 1999 after a complete

years' worth of data had been gathered. In light of the enormous quantity of material to be processed, priority was given to the identification of specimens from Doneguebougou. The results, to date, of cytotaxonomic identification of semi-gravid females and PCR identification of other specimens are shown in Figures 1 & 2, respectively. In both cases the proportions indicated are the monthly sample of a specific karyotype/species expressed as a proportion of the total catch identified for that karyotype/species.

ii. Other sites within Doneguebougou village. PSC sampling of sites such as abandoned houses, chicken coops, tree holes/termite mound, all within the confines of the village, continued in parallel with that of inhabited human dwellings (Figs 3. & 4).

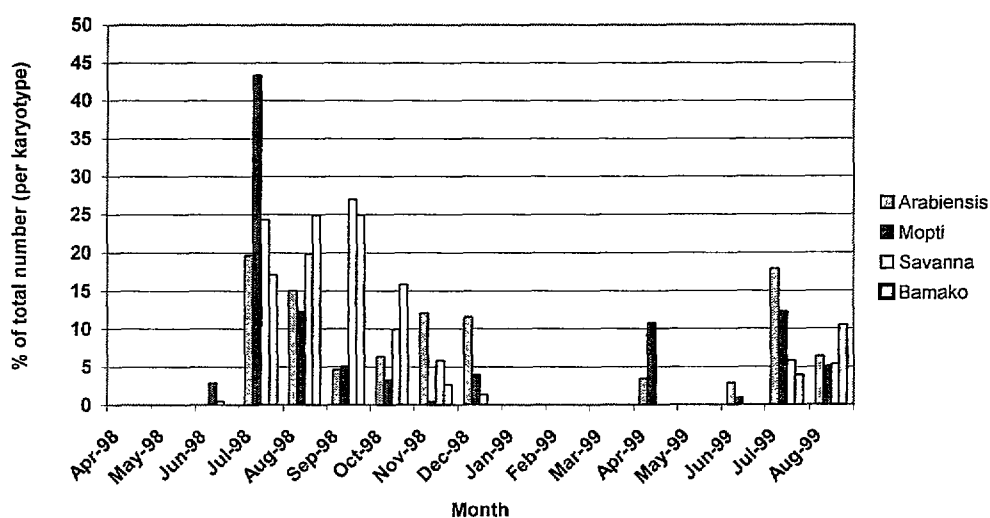


Figure 1. Composition of karyotyped semi-gravid *An.gambiae* s.l. caught by PSC from human dwellings in Doneguebougou April 1998 - Sept 1999.

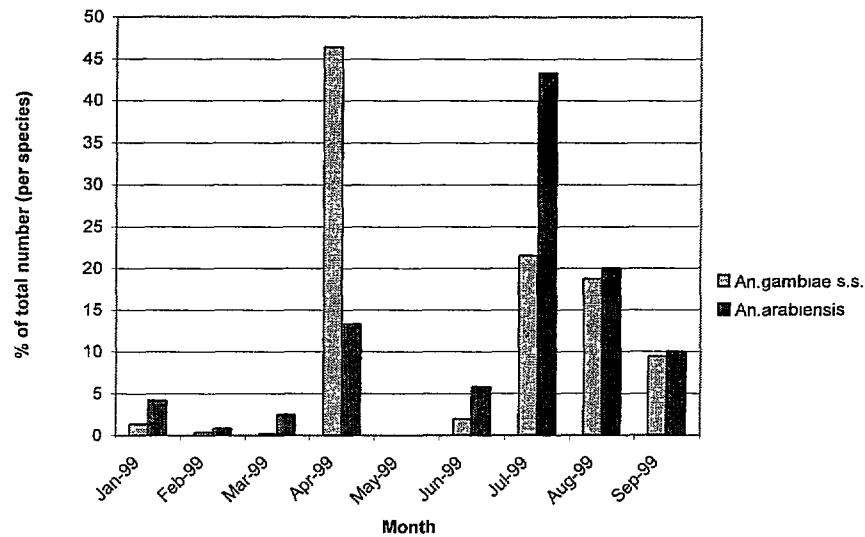


Figure 2. PCR identification of unfed, fed and gravid *An. gambiae* s.l. specimens caught by PSC in human dwellings – Doneguebougou.

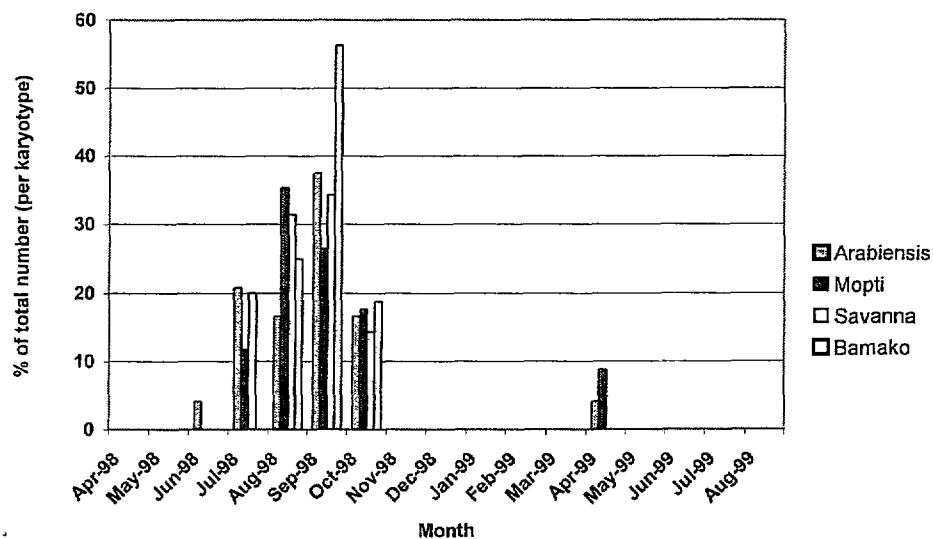


Figure 3. Composition of karyotyped *An. gambiae* s.l. caught by PSC in sites other than human dwellings in Doneguebougou April 1998 - Sept 1999.

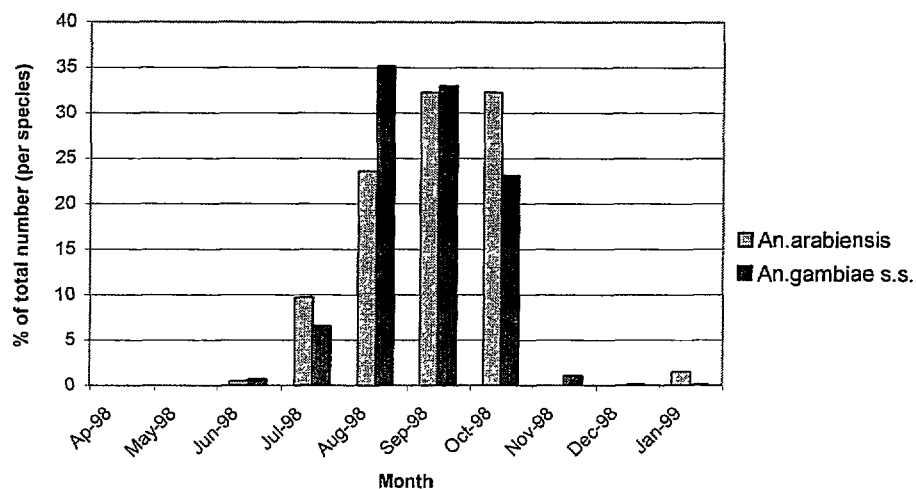


Figure 4. PCR identification of unfed, fed and gravid *An. gambiae* s.l. specimens caught in sites other than human dwellings – Doneguebougou.

B.1.2. Sampling of potential aestivation sites in the field.

i) **Termite mounds.** Five active termite mounds were selected along two transects: four along the northern transect (an area containing the majority of active mounds) and one on the eastern transects. The termite mounds ranged in size from 3x2m (length x width) to 4x4m. Cotton mesh emergence cages, 1.75m high, were constructed around the termite mounds using bamboo supports. A 1m zipper incorporated into one corner of each net allowed entry of collectors into a cage. Two black cotton squares (0.5x0.5m) sewn into the eastern facing corners of each cage provided an area of shade, reducing the exposure of any insects, which may have emerged to adverse morning conditions. The mounds were monitored each morning between 0600 – 0700h and any mosquitoes within were removed with a mouth aspirator and transferred to Carnoy fixative.

During the first week after installation a number of female *Culex* spp. were caught inside the largest of the emergence cages. These comprised one blood fed and 3 unfed individuals. No *An. gambiae* s.l. were observed in any of the cages.

ii) **Rodent burrows.** The 1998 field season had shown inverted funnel exit traps placed over the entrances to rodent burrows to be a feasible sampling method for this type of habitat. In 1999, efforts were concentrated on identifying and sampling from areas in which burrows were concentrated. Three such aggregations were located: one east of larval site 1, one SE of larval site 1 and NE of the village, at the base of the escarpment. Twenty-five exit traps in total were placed over rodent burrows in these areas. The traps remained in situ from May – August during which period the holding containers were inspected for mosquitoes each morning.

Although the exit traps caught many Pyschodids and a few early instar *Locusta* spp., no *An. gambiae* s.l. were trapped exiting these burrows.

iii) Malaise Traps. A number of Malaise traps were positioned around the village of Donegubougou in a attempt to sample potential aestivation sites which could not be sampled directly by active collecting or by exit traps. By intercepting mosquitoes flying on their way to breeding sites or the village we hoped to gain an indication of the presence of mosquitoes in certain target areas of the bush prior to the build up of the population in the village/larval sites.

Ten Malaise traps were designed and built in Mali. The traps were 3m in length, 2 m high at each end (1.5m at the center) with a 0.75m overhang to channel insects flying into the mesh towards collecting vessels located at either end. The traps were designed to allow entry of insects into the collecting vessels from either side. Each collection vessel contained 300ml of Carnoy's fixative. Heavy-duty nylon mesh was used in the construction of the traps, which were supported and held in situ by bamboo poles sunk into the ground. A ring of thorn branches, encircled each trap which had been placed across an animal trail to prevent cattle damage.

Two traps were placed NE of Donegubougou, intercepting natural channels of a rocky escarpment. A further 3 were placed to the east of the village, intercepting animal trails to and from larval site 1. Of the three Malaise traps to the west of the village, two were situated on animal trails near the riverbed of larval site 2 and the third positioned across a depression between two hillocks. One Malaise trap was situated near larval site 4 and last of the ten in the village of Banambani. The trap at Banambani, intercepted a trail from the area of permanent standing water to a small inhabited hamlet.

The Malaise traps were sampling flying insects from May – Sept 1999. Collecting vessels were checked daily (am and p.m.) for evaporation of fixative and catches emptied twice weekly at 3 & 4 day intervals.

Over the three - month period in which the Malaise traps were operating, three male *An. gambiae* s.l. and a number of *Culex* spp. were captured. One male was captured by the solitary trap in Banambani (June 1999)

iv) Human bait catches. Human bait catches (HBC) were repeated from April – July 1999 with the intention of observing any build up in the population of *An. gambiae* s.l. in certain target areas, prior to their occurrence in the village. The location of sampling sites in the bush was identical to those used in 1998 (N, E, S & W). Once again, parallel HBC's were conducted in two sectors of the village on the same night.

Two teams, each comprising of two collectors, were employed at each site; the first team collecting from 1800 – 0000 h, the second from 0000 – 0600h. The two bush sites sampled during the first HBC of each month were selected at random, thereby pre-selecting the other two sites for the subsequent nights catching for that replicate. The location of sampling sites in the two sectors of the village were also selected at random; in both sectors one member of a team collected inside a room, the other outside. No team remained in a particular site (bush/village) for more than one 6h period.

All mosquitoes landing on the exposed arms and legs of the collectors were sampled with mouth aspirators. Collections were separated into 2h time periods after which the collecting cups were changed. In each village site, the two collectors exchanged positions

(inside/outside) every two hours. All mosquitoes caught were held in humid conditions to ensure their survival until the end of the experiment.

Identification of the preliminary experiments in the season 1998-1999 indicated that all three female *An.gambiae* s.l. caught by HBC were *An.gambiae* s.s. Catches from the bush in season 1999-2000, are outlined in Table 1. The village catches in July comprised some 57.6% *An.gambiae* s.s. and 42.41% *An.arabiensis*. Bush catches have yet to be identified.

Table 1. Summary of HBC from Doneguebougou, dry/transition season 1999. Catches are totals for two sites (bush) and two areas in the village (2 capturing stations each site).

Month	Bush Sites		Village Sites	
	<i>An.gambiae</i> s.l.	Other <i>Anopheles</i> spp.	<i>An.gambiae</i> s.l.	Other <i>Anopheles</i> spp.
April	1	7 <i>An.pharoensis</i>	0	1 <i>An.funestus</i> 1 <i>An.pharoensis</i>
May	0	2 <i>An.pharoensis</i>	0	1 <i>An.pharoensis</i>
June	0	0	0	0
July	5	0	60	2 <i>An.pharoensis</i>

vi) **Mark-release and recapture - longevity experiments: Doneguebougou & Banambani.** Mosquitoes were captured for marking in the two villages over a two-week period in September 1999. A total of 394 rooms were sampled, using mouth aspirators, in Doneguebougou resulting in a total catch of 4770 female *An. gambiae* s.l. These were marked with red fluorescent dye and released near to their sites of capture. A similar collecting program in Banambani (210 rooms) allowed the marking of 4595 *An. gambiae* s.l. with blue marker. During the initial capture period some movement of mosquitoes between the two villages was observed; two red mosquitoes (marked in Doneguebougou) were captured in Banambani and two blue mosquitoes were captured in Doneguebougou.

It is intended to run this study through the dry season, with the first specific recaptures of marked mosquitoes in both villages being made in December 1999. This recapture will be carried out in all inhabited dwellings in each village using mouth aspirators. Each village will be sampled monthly. It is envisaged that the final recapture in April 2000 will be a PSC and at this time additional PSC's will be performed in other neighboring villages. This should establish whether there has been any dispersal of the marked population and may indicate the presence of localized, favorable habitats. Any marked mosquitoes recaptured will be identified by cytotaxonomy (half-gravid) and by PCR-RFLP for other abdominal stages. All half-gravid mosquitoes will be stored in Carnoy's solution and other stages will be stored in 70% ethanol.

A parallel study was also sampling the population of *An. gambiae* s.l. in Doneguebougou during the transmission season (June- December) and PSC's conducted as part of this study in October captured 13 marked mosquitoes: 11 red and 2 blue. However, in

Banambani the population of released mosquitoes had not been sampled until the first recapture in December 1999.

Re-captures are currently taking place at monthly intervals, with the same number of dwellings being sampled in each village as were used in the initial capture. Mouth aspirators are being used to sample mosquitoes. To date no marked *An. gambiae* s.l. have been recaptured.

v) Tree Holes, Wells & Rock Crevices. During 1998, tree holes within a 800m radius from the center of both Doneguebougou and Banambani were sampled monthly by PSC. The purchase of a back - mounted aspirator in 1999 enabled a period of intensive sampling to be performed at the end of the dry season during which a number of trees with previously inaccessible holes/hollows were also sampled. The equipment allowed rapid and thorough sampling from a number of other potential sites which it had not been possible to sample from e.g. wells, small rock holes/crevices.

Table 2. shows that no female *An.gambiae* s.l. were caught from these sites, although they appear to be suitable refuges for other species, particularly *Culex*. In the light of these data and year round sampling of tree holes in both villages, it would appear that the significance of tree holes and wells as potential aestivation sites is limited. As yet insufficient collections have been made from rock crevices throughout the dry season to confirm that this is also the case for these sites.

vi) Automated temperature and humidity measurements. In July 1999 three Hobo^R data loggers were installed in Doneguebougou to provide detailed, specific information on the prevailing environmental conditions. One was positioned in a open area of the bush north of the Dispensary. The second was attached to the roof beam of a human dwelling ("terrace" roof type) in the center of the village. The third was positioned on a Mango tree in the small grove to the south of the village.

The sampling frequency of each logger is set at 1 min with the hourly mean being recorded. By recording temperature and humidity from these sites we will gain invaluable information on what we feel are the two major environmental influences on aestivation. In addition to providing a possible correlation of environmental conditions with field catches, the data will also help to recreate "field conditions" for future laboratory studies in which we will elucidate the influences of photoperiod, temperature and humidity on aestivation. The day and night means of both temperature and humidity recorded during the reporting period from each instrument have been calculated and are shown in Figs. 5-7.

Table 2. Summary of back-mounted aspirator catches from potential aestivation sites in the bush surrounding Donegubougou and Banambani villages. Number sampled = sum of both villages.

	Number sampled	<i>An. gambiae</i> s.l.	Other Anophelines	Other Culicidae
April 1999				
Tree holes	108	1 male		>100 <i>Culex</i> spp.
Wells	16		1 <i>An. pharoensis</i>	>100 <i>Culex</i> spp.
Rock holes/crevices	75			1 <i>Culex</i> spp.
May 1999				
Tree holes	187	2 male	3 <i>An. rufipes</i>	>100 <i>Culex</i> spp
Wells	45	2 male		>100 <i>Culex</i> spp
Rock holes/crevices	34	3 male	1 <i>An. rufipes</i>	12 <i>Culex</i> spp.
June 1999				
Tree holes	64	0		
Wells	45	0	2 <i>An. rufipes</i>	>100 <i>Culex</i> spp.
Rock holes/crevices	6	0		
July 1999				
Tree holes	20	0	0	0
Wells	7	0	0	0
Rock holes/crevices	6	0	0	0

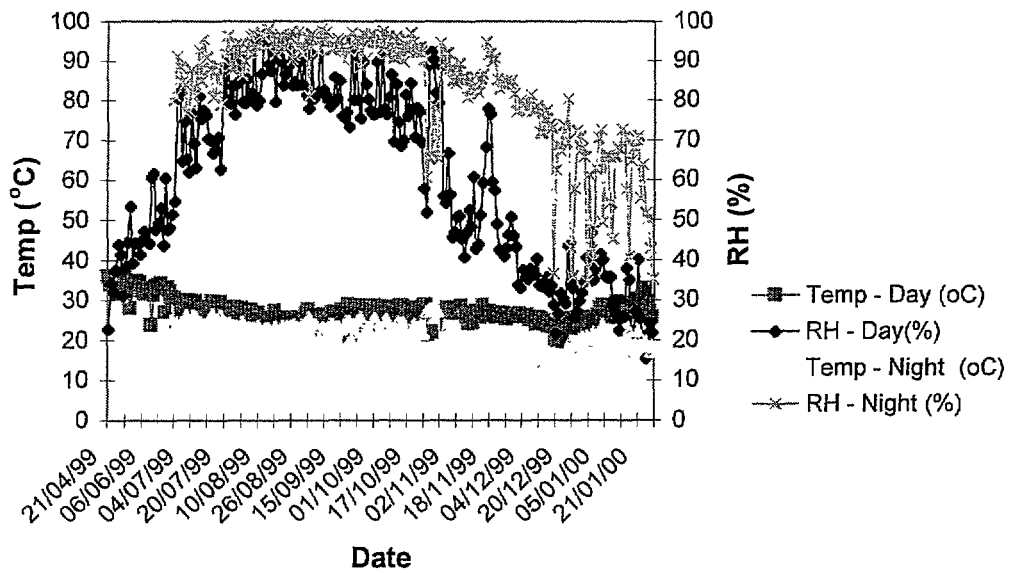


Figure 5. Fluctuation in day and night temperatures ($^{\circ}\text{C}$) and RH (%) recorded within human dwelling, Doneguebougou.

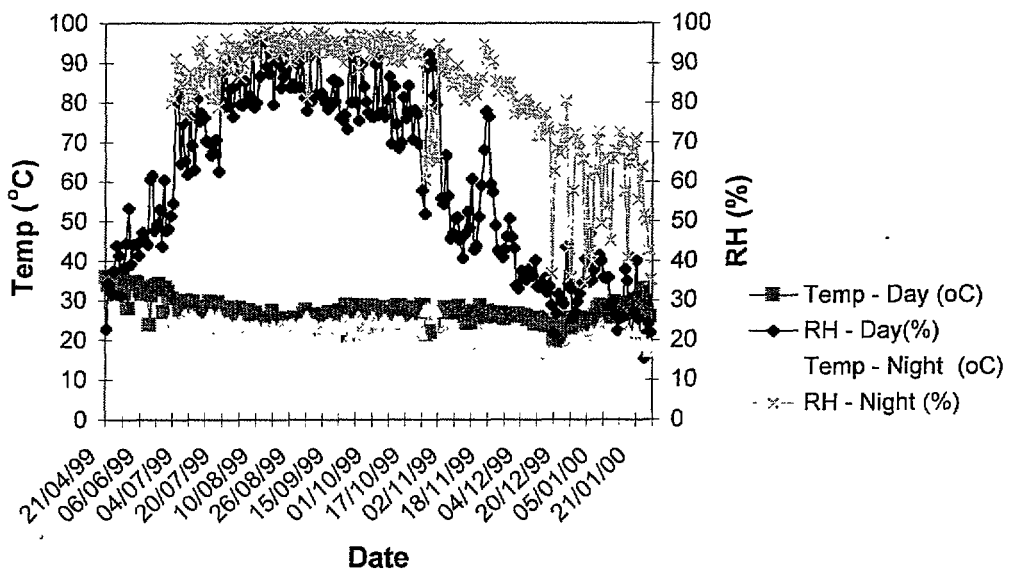


Figure 6. Fluctuation in day and night temperatures ($^{\circ}\text{C}$) and RH (%) recorded within Mango grove, Doneguebougou.

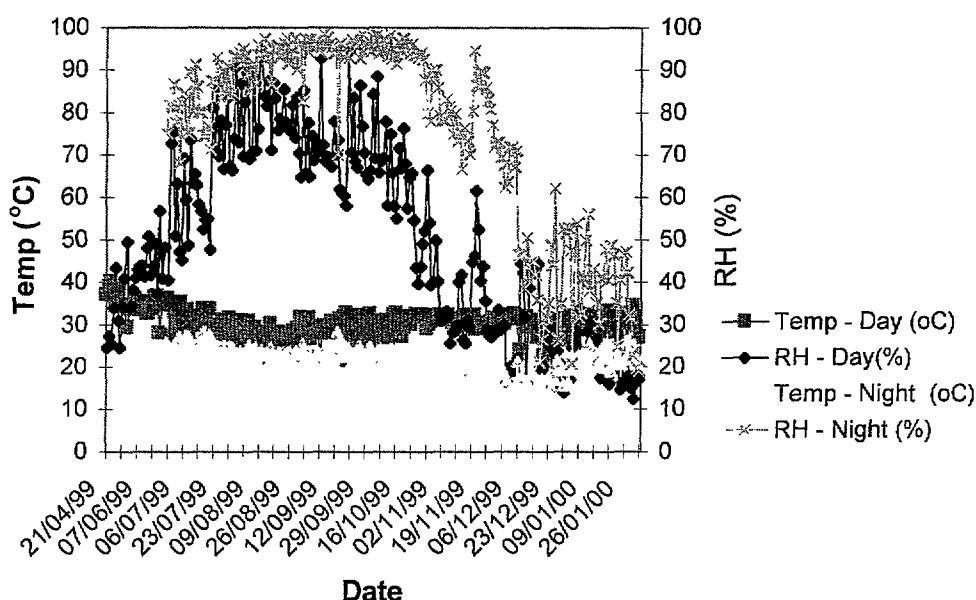


Figure 7. Fluctuation in day and night temperatures ($^{\circ}\text{C}$) and RH (%) recorded from the bush, Doneguebougou.

B.1.3. Summary – Mali

The failure to successfully trap aestivating *An. gambiae* from the variety of sites investigated thus far is clearly not due to the application of inappropriate sampling techniques. In every case we have shown the efficacy of the sampling method by obtaining catches of other hematophagous insects. For example the capture of *Culex* mosquitoes from a termite mound indicates an appropriate technique was used and more importantly indicates that these sites are certainly used by *resting* mosquitoes.

A low-level monitoring programme will continue during the dry season 1999-2000. This will involve PSC sampling in village sites and aspirator catches in the bush. We will also initiate alternative methods to investigate the phenomenon.

1. The existence of laboratory colonies of all three chromosomal forms of *An. gambiae* s.s. in Mali is a crucial step forward as it will allow us to initiate studies to induce aestivation in the laboratory. We are currently designing small C.T. chambers which will be used in Mali to assess the interactions of temperature, humidity and photoperiod on the biology of *An. gambiae* s.s.
2. We were unable to identify any caves or caverns in the vicinity of Doneguebougou or Banambani. This remains the one potential aestivation site that we have not yet sampled. We intend this year to locate and sample any caves in the Kati Circle.

B.2. Research Accomplishments - Israel

B.2.1. Respiratory Physiology. Following on from our success in developing an open or flow-through system for measuring respiratory rates of mosquitoes we now report on the changes in mosquito metabolism which occur after blood feeding. We have also shown, for the first time, the energetic costs of parasitaemia on the mosquito. Currently, we are investigating the effects of hypoxic environments on the longevity and fecundity of *An.gambiae* (G-3) and whether such conditions induce a lowering of metabolic rate.

Results. Female *Ae.aegypti* maintained on a 10% sucrose diet *ad lib.* show an age dependent decrease in oxygen consumption (Fig. 8.). When a similar aged cohort are fed chicken blood from an uninfected chicken VO_2 increases rapidly to peak 48 hours post meal. This metabolic peak coincides with peak peptidase activity and hence digestion of the blood meal. The high level of metabolism is maintained during ovarian development, highlighting the high energetic costs to the female, and is significantly lower only on day 6 (144 hours) post feeding.

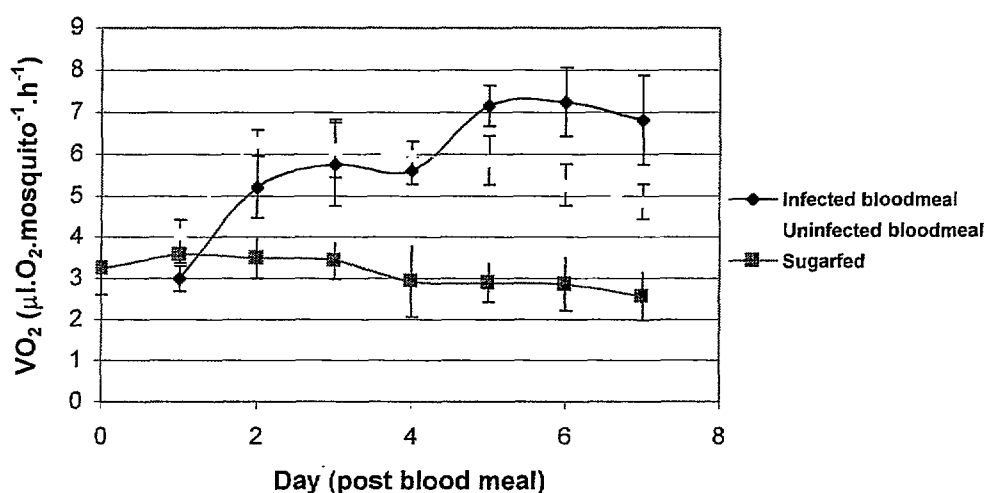


Figure 8. Oxygen consumption rates ($VO_2 \pm SD$) of female *Ae.aegypti* fed *P.gallinaceum* infected and uninfected chicken blood.

Feeding females on chickens with 30-35% *P.gallinaceum* parasitaemia produces a similar VO_2 curve for the first 3 days post blood meal, however, on day 5 post blood meal there is a further significant increase in VO_2 compared to the earlier digestive activity peak (Fig.8). Although this increased metabolic activity diminishes with time, it remains higher than that in similar aged mosquitoes fed uninfected chicken blood.

In order to elucidate whether this pattern of increase in metabolic activity was in some way parasite induced, two groups of chicken blood (uninfected and *P.gallinaceum* infected)

were subjected to freeze-thaw cycling to kill parasites. Blood smears revealed that no viable parasites remained in the sample of infected chicken blood. Female mosquitoes were then presented this blood via a membrane feeder. Oxygen consumption curves of those females that fed to repletion were subsequently produced (Fig 9.). There was no significant difference between the two profiles produced, suggesting that the increase in metabolic activity recorded with infected blood was parasite induced.

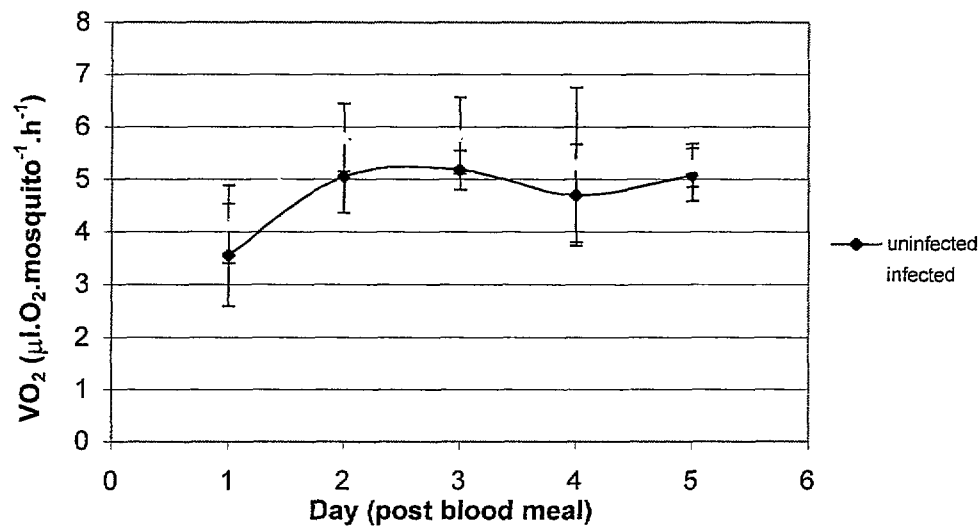


Figure 9. Oxygen consumption rates ($VO_2 \pm SD$) of female *Ae. aegypti* membrane- fed infected (*P. gallinacaeum*) and uninfected chicken blood subjected to "freeze- thaw" cycling.

B.2.3. Future studies

Ongoing experiments in Israel are using these respiratory physiology techniques to resolve the question of the effects of hypoxia on the metabolism, longevity and fecundity of *An. gambiae*. The effects of decreasing O₂ concentrations and increasing CO₂ concentrations will be assessed independently of each other. In addition mosquitoes will be reared in atmospheres mimicking the composition of O₂ and CO₂ that we have found in rodent burrows in Mali.

C. Scientific Impact of Collaboration

C.1. Visit of Israeli team to Mali.

With attempts to locate mosquitoes during this reporting period focussing on the transition period from dry to wet season, Dr A Bhasin made an extended visit to Mali to

coincide with this period (April – July 1999). Whilst in Mali, Dr Bhasin worked closely with Adama Dao, his Malian colleague, on the technical and practical aspects of the study. Field trips to Doneguebougou of 7-10 days duration were made twice monthly during the period of Dr Bhasins' stay. Dr Alon Warburg spent 2 weeks in Mali during June to participate in field work, assess the progress of the sampling regime and conduct discussions with Y. Toure and the Malian scientists.

C.2. Visit of Adama Dao to Israel.

Mr Adama Dao made his second visit to Israel from 15th November – 16th December 1999. With significant progress being made in Mali towards completing the identification of mosquito samples caught thus far, it was imperative to collate the data and design a working database that could be used by both teams. This database was successfully set up and has allowed for an immediate transfer of data between the two laboratories. In addition, Mr Dao assisted greatly in the formulation of plans for the next season's field studies and his expertise was invaluable in the design of protocols for future laboratory studies which will be conducted under his supervision in Mali.

D. Description of Project Impact

1. Increased awareness of the problematics of aestivation of malaria vectors during the dry seasons.
2. Training of Adama Dao in Israel has enabled us to proceed with data processing at an enhanced rate.

E. Strengthening of developing country institutions

1. Training of Dr L. Araya in Israel has led to establishment of a facility for immunological assessment of human serum samples and active cases collected in the areas described.
2. The acquisition of a powerful computer has facilitated the establishment of a data base for collating data from endemic regions.

F. Future work

1. Completion of the aestivation shelter survey.
2. Completion of the respiratory physiology studies including respiration of Malaria infected mosquitoes.

SECTION 2

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| A. Managerial issues | None |
| B. Budget | None |